O-43

Implication of Dystrophin Hinge Regions in Micro-Dystrophin Gene Replacement Therapy for Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked muscle disease resulting from the loss of cytoskeletal protein dystrophin. The dystrophin gene is one of the largest genes in the human genome. Dystrophin protein consists of four regions; amino terminus (NT), central rod domain with 24 spectrin-like repeat (R) regions and four hinge (H) regions, cysteine rich domain (CR) and carboxyl- terminal domain. While the biological functions of NT, CR and CT as well as repeats in the rod domain and H2 and H3 have been extensively interrogated, little is known about the function of H1 and H4. Using adeno-associated virus (AAV) delivery as a platform, we studied the structure-function relationship of H1 and H4 in the context of micro-dystrophin. We started with a microgene carrying both H1 and H4. A series of new microgenes with full or partial H1 and/or H4 deletion were generated. All microgenes were delivered to the tibialis anterior muscle of 3-m-old male mdx4cv mice using AAV-9 at the dose of 1E12 viral genome particles/muscle. Muscle force was evaluated at 6-month post injection. Compared with the parental construct, complete deletion of H1 did not alter micro-dystrophin function, whereas complete deletion of H4 compromised force rescue by micro-dystrophin. Some partial deletion constructs yielded better muscle force rescue while others showed similar rescue as that of the parental construct. These data suggest that H1 and H4 play an important role in muscle function. (Supported by NIH and Jackson Freel DMD Research Fund).